CORRELATION OF DIFFERENTIAL THERMAL ANALYSIS DATA WITH THE SHRINKAGE TEMPERATURE OF COLLAGEN AND LEATHER[1]

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ABSTRACT

Shrinkage temperature data obtained on suspended specimens of raw hide substance and a variety of commercial leathers are compared with values obtained by measuring the thermodynamic changes using differential thermal analysis. In every instance the correlation between the data of these two methods was statistically highly significant, and it is concluded that they measure the same basic phenomenon. It is suggested that the absolute values for the phase transition measured by DTA be used as the reference for the thermal behavior of collagen and its products. Use of suspended specimens offers a simplified procedure, which could be defined as a method for the measurement of shrinkage temperatures. The use of pressurized equipment permitted the extension of the temperature range to include products shrinking above the boiling point of water (100°C.).

INTRODUCTION

The hydrothermal shrinkage of collagen and its products is a characteristic property that has found wide application in research and production control. Although the phenomenon has been studied by numerous workers [see Gustavson (1) and Nayudamma (2)], yet its physical-chemical nature has not been fully explained. This behavior has been variously described as due to degradation, denaturation, rearrangement, gelatinization, or various combinations of these processes. A more logical explanation has been that shrinkage is a manifestation of the melting or fusion of the crystalline regions in the collagen molecule. The application of the term "melting" to the shrinkage of collagen is not new. As early as 1932 Wöhlisch (3) considered the transformation as such a phase transition. More recently, Wiederhorn (4) and coworkers discussed the shrinkage

of collagen from this point of view. Additional evidence that lends support to this theory is found in other published works. For example, Astbury (5) observed that the characteristic x-ray diffraction pattern of crystalline collagen disappeared on shrinkage. Theis (6), Kutyanin (7) and Lennox (8), found that the shrinkage temperature depended on dilution. Garrett and Flory (9) investigated this transformation and observed that tendon collagen containing ethylene glycol abruptly increased in volume at a specific temperature for each glycol content. Witnauer and Fee (10) explored the effect of diluents on the shrinkage of tanned and untanned cowhide and found the behavior consistent with phase transitions. More recently Witnauer and Wisnewski (11) studied the thermal behavior of collagen (hide) and leather by applying differential thermal analysis (DTA) during heating. These studies revealed that shrinkage was accompanied by absorption of heat as would be expected from an endothermic process such as fusion or melting.

Although the shrinkage temperature of collagen products is the most widely used test for leathering and degree of tannage, yet scientists are at odds concerning an accepted procedure for obtaining precise results. Numerous studies have demonstrated that the temperature of shrinkage is affected to an appreciable extent by the tension applied to the specimen (12) and the composition of the heating medium (13). Difficulty usually was experienced in reproducing results due to friction within the apparatus and other factors.

A recent collaborative study (14) indicated that acceptable interlaboratory agreement could be obtained using freely suspended samples. It was of interest to determine whether the values obtained by this technique are significantly related to the melting or fusion temperature of the collagen molecule. The availability of instrumentation for determining the absolute temperature of fusion through differential thermal analysis now makes such a study possible. The comparison of results obtained by the two procedures is the subject of this report.

EXPERIMENTAL

Instrumentation.—The DTA instrument used in this investigation was the duPont 900 Differential Thermal Analyzer.[3] The experimental techniques which were developed for use of DTA in measuring shrinkage temperature and the interpretation of thermograms have been discussed previously (11). For the reported measurements the rate of heating was 5.0°C./min.; the differential temperature scale was set at a sensitivity of 0.1°C./in., and the temperature scale was set at 10°C./in. The temperature range was from about 30°C. to about 130°C. For measurements greater than 95°C. a specially constructed pressure cell was used (15). For standardization, a National Bureau of Standards' benzoic acid was used as the DTA reference, which gave a peak temperature of 122.8°C.

A complete description of the equipment involved for determining shrinkage temperature using suspended specimens was published recently (14). The heating of the bath was adjusted to give a rise of 3–4°C. per minute, and all measurements were made in distilled water. Heating was started from about 30°C. and continued until the first discernible shrinkage was observed. The temperature was also noted after the specimen had shrunk to marks on the holder corresponding to five percent and to ten percent of its length. Heating was continued for an additional minute or two to establish that ten percent was not the limit of the specimen for shrinkage.[4]

Sample preparation.—A selection of hides and leathers was made to insure a comparison of different temperatures in the range from about 60°C. to about
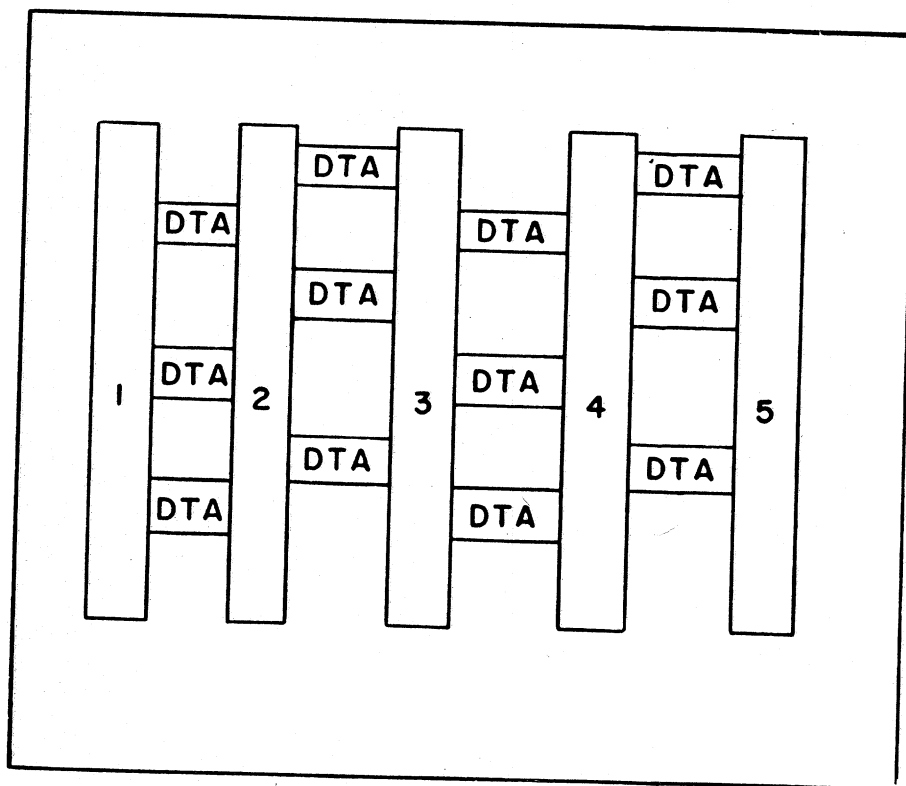

FIGURE 1.—Sampling scheme for selection of specimens. Locations marked 1–5 are ¼ by 2-¼ inch specimens for determining the shrinkage temperature under freely suspended conditions, and the areas marked DTA represent locations from which random specimens were taken for differential thermal analysis. A typical specimen was about 5 mm. x 10 mm. and weighed approximately 20–30 mg.

[4]The standard deviations between replicates varied as follows: initial shrink from 0.55–3.31 with 24 values under 2.0; 5% shrink from 0.22 to 2.60 with 22 values under 0.99; and 10% shrink from 0.19 to 1.20 with 22 values under 0.85.

120°C. Care was taken to make this comparison under essentially identical conditions and to minimize the effect of uncontrollable sources of variability in the two methods which could lead to discrepancies in the reported values. The specimens for each comparison were removed from a unit area of a hide or leather, as shown in Fig. 1. Locations 1, 2, 3, 4 and 5 represent specimens used for shrinkage temperature determinations by the suspension method. Specimens for the DTA measurements were taken randomly from the locations marked DTA. Since preliminary tests using up to 15 replications showed a high degree of reproducibility between replicates, the number of specimens was finally reduced to four.[5] To insure identical wetting, each specimen was wet back in a beaker of distilled water in a vacuum desiccator. A vacuum was pulled to about 30 mm. Hg for one minute and then released. After three such cycles, the DTA measurement and the shrink temperature were determined immediately.
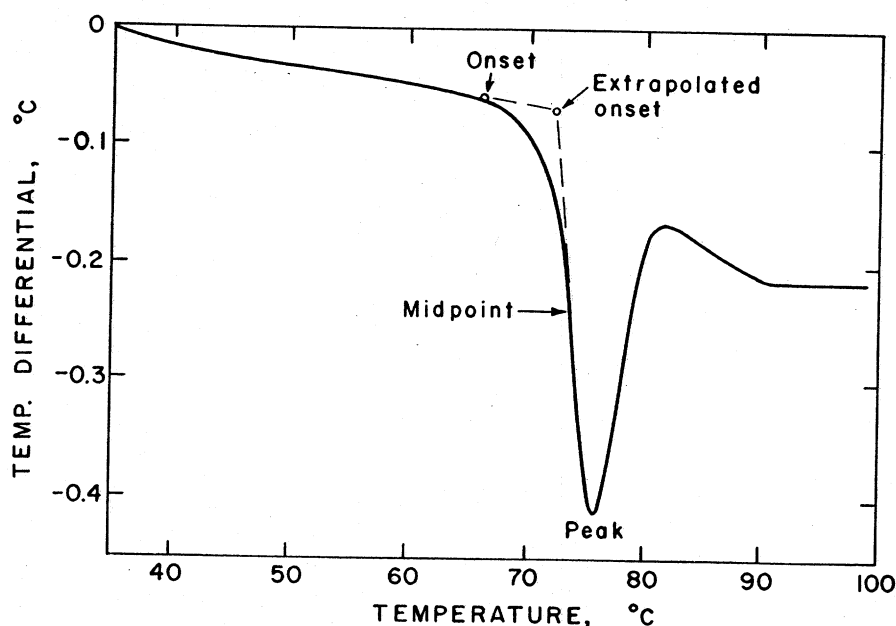

FIGURE 2.—DTA thermogram for a vegetable-tanned unfinished calfskin immersed in water.

Typical leather-water thermogram.—The thermogram of a vegetable-tanned calfskin immersed in water is shown in Fig. 2. The temperature range studied was from 35°C. to 100°C., using a heating rate of 5°C./min. The thermo-

[5]The standard deviation between replicate observations varied as follows: approximate onset from 0.50–2.50 with 22 values under 1.07; extrapolated onset from 0.0–2.06 with 22 values under 1.17; midpoint from 0.06–1.52 with 23 values under 1.00; and peak from 0.08–1.11 with 22 values under 1.00.

TABLE I

DATA FROM DTA AND SUSPENSION SHRINKAGE TEMPERATURE MEASUREMENTS

No.	Sample[1] Description	DTA Thermogram Data[2]				Suspension Ts[3]		
		Approximate Onset °C.	Extrapolated Onset °C.	Midpoint °C.	Peak °C.	Initial Shrink °C.	5% Shrink °C.	10% Shrink °C.
1	Chrome-tanned cowhide grain, after splitting	104.5	107.9	109.8	111.8	107.1	109.7	111.1
2	Chrome-tanned cowhide grain, after vegetable retan	106.0	109.5	112.7	116.2	108.9	113.0	115.9
3	Chrome-tanned, vegetable-retanned cowhide grain after fat-liquoring	106.0	109.5	112.9	116.2	110.3	114.4	116.8
4	Chrome-tanned cowhide grain, after splitting	99.4	101.9	103.4	105.0	100.2	102.7	103.2
5	Chrome-tanned cowhide grain, after glutaraldehyde retan	108.4	111.3	112.8	114.2	107.1	111.0	112.7
6	Chrome-tanned, glutaraldehyde retanned cowhide grain, after fatliquoring	106.0	109.0	111.1	113.2	102.0	108.7	110.2
7	Chrome-tanned side upper, HH weight leather	100.6	104.0	106.2	108.4	103.3	105.9	106.9
8	Chrome-tanned kip leather	100.0	103.0	105.4	107.8	98.8	103.3	104.8
9	Chrome-tanned white side upper leather	96.2	98.0	100.2	102.4	97.2	100.3	101.2
10	Chrome-tanned, glutaraldehyde retanned side upper leather	95.8	98.5	100.5	102.5	99.6	102.0	103.3
11	Double chrome-vegetable retanned mechanical leather	106.7	109.2	111.2	113.1	99.0	107.8	112.1
12	Chrome-tanned calf leather	97.0	100.0	101.6	103.1	92.9	102.1	102.8
13	Chrome-tanned white side upper leather	96.8	99.9	102.2	104.6	99.3	103.0	103.7
14	Chrome-tanned white side upper leather	90.5	92.1	93.6	95.2	92.5	94.5	95.4

TABLE I (Continued)

DATA FROM DTA AND SUSPENSION SHRINKAGE TEMPERATURE MEASUREMENTS

		DTA Thermogram Data[2]				Suspension Ts[3]		
	Sample[1]	Approximate Onset °C.	Extrapolated Onset °C.	Midpoint °C.	Peak °C.	Initial Shrink °C.	5% Shrink °C.	10% Shrink °C.
No.	Description							
15	Chrome-tanned calf leather	98.0	100.0	101.8	103.5	93.3	100.4	101.4
16	Vegetable-tanned domestic sheepskin (after tanning)	75.8	78.9	80.8	82.6	75.9	78.4	79.4
17	Vegetable-tanned sheepskin, hat band leather (crust stage)	76.3	80.0	81.6	83.2	76.9	80.1	80.9
18	Vegetable-tanned sheepskin, hat band leather	74.8	77.1	79.9	82.5	75.3	78.7	79.4
19	Chrome-tanned domestic sheepskin (after tanning)	110.0	112.3	113.5	114.5	110.4	112.9	114.1
20	Chrome-tanned sheepskin, slipper leather (in crust stage)	108.7	111.5	113.8	116.1	107.9	113.1	113.9
21	Chrome-tanned sheepskin, slipper leather	110.3	113.8	114.9	116.0	109.1	114.6	115.3
22	Vegetable-tanned, Morocco goat bookbinding leather	77.8	79.3	81.6	84.0	71.0[4]	80.9	81.8
23	Chrome-tanned calf leather	81.8	84.5	86.7	88.9	62.1[4]	80.4	83.0
24	Chrome-tanned calf leather	81.8	84.3	85.7	87.1	65.5[4]	85.3	86.0
25	Depickled cabretta	59.0	61.6	63.1	64.6	59.7	61.4	62.0
26	Depickled cowhide grain	60.5	62.5	64.0	65.5	60.5	63.0	64.0

[1]All samples represent commercial production.
[2]Data for samples 1–9 are averages of eight determinations; all others are averages of four determinations.
[3]Temperatures corrected for thermometer stem exposure; all data are averages of five determinations.
[4]Shrinkage began so slowly that observation was uncertain.

gram shows a pronounced minimum with a peak at about 76°C., indicating a relatively large absorption of heat by the leather-water system. Visual observations made on the leather specimen during heating in the DTA apparatus revealed that shrinkage was taking place in the temperature range of the minimum. In discussing the data obtainable from thermograms, reference will be made to the following points of temperature:

(a) **Onset temperature:** The sample temperature at which the slope of the thermogram first departs from the base line on heating. This corresponds to the first indication that a change in the sample is starting to take place. Unfortunately, the exact position of the onset is often difficult to determine accurately. (Reported to nearest degree.)

(b) **Peak temperature:** The sample temperature at which the temperature differential between sample and reference is greatest.[6] The transformation taking place in the sample is almost complete at this temperature. For compounds undergoing fusion, the temperature at this point is reported as the compound's melting point. (Reported to nearest 0.1°C.)

(c) **Extrapolated onset temperature:** The temperature obtained by extrapolation of the base line and the straight line portion on the low temperature side of the peak. This point represents the starting temperature for the major portion of the transformation. (Reported to nearest 0.5°C.)

(d) **Midpoint temperature:** This was the arithmetic mean of the extrapolated onset and peak temperatures.

DISCUSSION OF RESULTS

The study was conducted with 26 samples, representing tanned and untanned cattlehides and sheepskins. Except for samples 22, 23 and 24, all were procured from recent production, and samples representing wet-end operations (samples 1–6, 16, 19, 25, 26) were kept moist by wrapping in plastic sheets. The samples represent a cross-section of commercial tannages and include chrome leathers, chrome retanned with vegetable, chrome retanned with glutaraldehyde, and vegetable tanned leathers. The data obtained are presented in Table I.

A cursory examination reveals that there is appreciable agreement between the parameters taken from the DTA thermograms and those obtained with shrinkage measurements on suspended samples. It is interesting to note that DTA readings at the approximate onset detected an early change with 14 samples while the

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[6]The direction of the deviation from the base line indicates whether the heat effect is exothermic or endothermic. In DTA the resulting curve is called a peak regardless of sign.

initial shrinkage on the suspended samples detected the primary change with 11 samples (one sample gave an identical reading by both methods). The extrapolated onset gave higher readings than the initial shrinkage in the majority of cases (23 samples). Some difficulty was experienced with samples 21, 22 and 23 in detecting the start of shrinking and as a result these samples showed the greatest variation between the two methods. Readings at five percent and ten percent shrinkage showed rather close agreement to those obtained with DTA at the extrapolated onset, midpoint and peak.

To determine the relationship between these parameters and their true significance, the data were analyzed statistically. The results of the statistical analyses are presented in Table II. In each of the 12 cases the "F" value (column 2)

TABLE II

SUMMARY OF STATISTICAL ANALYSIS

Variable	F	r	$t_{.05S}$	a	b
Approx. onset vs. initial shrink	199**	0.944	±11.9	− 8.1	1.069
Extrap. onset vs. initial shrink	205**	0.946	±11.6	− 9.8	1.056
Midpoint vs. initial shrink	211**	0.948	±11.5	−11.4	1.051
Peak vs. initial shrink	209**	0.947	±12.5	−12.5	1.042
Approx. onset vs. 5% shrink	1772**	0.993	± 3.87	− 0.35	1.044
Extrap. onset vs. 5% shrink	1999**	0.994	± 3.65	− 1.9	1.030
Midpoint vs. 5% shrink	2360**	0.995	± 3.36	− 3.3	1.025
Peak vs. 5% shrink	2217**	0.995	± 3.47	− 4.5	1.016
Approx. onset vs. 10% shrink	1972**	0.994	± 3.74	− 0.87	1.064
Extrap. onset vs. 10% shrink	2322**	0.995	± 3.45	− 2.4	1.050
Midpoint vs. 10% shrink	3367**	0.996	± 2.87	− 4.1	1.045
Peak vs. 10% shrink	3767**	0.997	± 2.71	− 5.4	1.038

**Significant at 1% level.
r = Coefficient of correlation.
$t_{.05S}$ = 95% confidence limits of a predicted value at $X_1 = \overline{X}$.
a = $\overline{Y} - b\overline{X}$, i. e., the intercept of the Y axis.
b = the regression coefficient, i. e., the slope of the line.

is considerably larger than 7.82, the value for $F_{.01}$. Thi smeans that the probability that these are real relationships, and not simply due to chance, greatly exceeds p = .99. Thus, in more than 99 cases out of 100 you would expect to be correct in stating that these are significant relationships. The third column shows the coefficient of correlation which measures the magnitude of the relationship between each set of values. As the maximum correlation coefficient is equal to 1.0, it can be concluded that all 12 correlations are not only highly significant

but also quite large. This would be the expected result if the two methods were measuring the same phenomenon.

The fourth column presents the 95 percent confidence limits of a predicted value of shrink temperature. The prediction equation is $\hat{Y} = a + bX \pm t_{.05}S$, and the values for Y intercept a and the slope b are given in columns four and five. In using this equation all that is necessary is to substitute a DTA value for X and solve for the predicted value of shrink temperature, $\hat{Y}$. The true shrink temperature would be expected to be within $\hat{Y} \pm t_{.05}S$, 95 percent of the time. Thus, in actual practice, the ten percent shrinkage temperature would be within 2.71 degrees 95 percent of the time and within 1.31 degrees approximatly 70 percent of the time from the DTA peak temperature value.

It has been determined statistically that the two methods are measuring the same phenomena. The question remains how best to interpret the shrinkage behavior of leather as measured on suspended samples (initial shrinkage, 5 percent shrinkage and 10 percent shrinkage) in the light of evidence that can be extracted from the four points of reference on the DTA thermograms (approximate onset, extrapolated onset, midpoint and peak temperatures). Consideration that the shrinkage temperature of leather is a melting phenomenon requires that an endothermic process be measurable during the shrinkage. Unlike a pure compound which melts over a narrow range, thermograms for leather and hide substance show that the endothermic process takes place over a range. In these studies the observed endotherm covered a range from 4.7° to 10.2°C., although leathers have been tested that covered a range as high as 16°C. The temperature range observed is influenced by the type of tannage (chrome, vegetable or combination), the uniformity of tannage, and undoubtedly other factors in the history of the leather.

In a broad thermogram the change in slope at the onset is usually so gradual that it is difficult to estimate the exact point of onset. The precision of readings for the onset can be improved by extrapolating the base line and the straight line portion of the endotherm, but the reproducibility of this extrapolated onset between replicates still leaves much to be desired. The peak temperature, however, can be determined with the highest precision. Since this is the point at which the reaction is essentially complete, it is not surprising that the recorded peak temperatures exceeded the shrinkage temperatures obtained with the suspended specimens. It was only when the suspended specimens were permitted to shrink to ten percent of their length that the temperatures approached those of the peak temperature, and three samples actually exceeded the peak temperature by a small margin. Extending this reasoning will lead us to the ultimate conclusion that the peak temperature would coincide with the complete shrinkage of the specimen. Unfortunately, instrumentation is not available for making this latter determination with precision.

It seems that a more logical approach would be to apply the basic definition for the melting point of an organic chemical. This is usually considered as the temperature at which the melt is in equilibrium with the solid, or when half of the material has melted. On the thermogram this should correspond to the midpoint between the onset and the peak. Calculation of the confidence limits at the five percent level indicate that temperatures at ten percent shrinkage reflect more closely the midpoint and peak temperatures from the DTA thermograms; yet from practical considerations, the deviations predicted for temperature readings at five percent shrinkage could be tolerated.

The intercept of the best lines fitted by least squares to the data indicate a slight bias in favor of the DTA reading. The negative values for the intercept probably signify a lag in detection of the shrinkage. Evidently a certain amount of melting occurs before recognizable shrinkage manifests itself. Also these early stages are undoubtedly obscured by the initial swelling of leather, the relaxation of stresses, and other factors. Weir (16) observed an increase in volume of the leathers when heated in water with a significant increase at the transition phase accompanying shrinkage, while Shaw and Maeser (17) measured these transitions as changes in buoyancy.

CONCLUSIONS

In accordance with the statistical results, there is no question that the shrinkage of leather is a manifestation of the melting of the hydrated crystalline regions complicated to a degree by the fibrous nature of the structure and the presence of tanning agents and other substances. This thermal transition can be measured by means of differential thermal analysis. The correlation of DTA data with shrinkage temperature measurements on suspended specimens can form a basis for developing a meaningful definition of shrinkage temperature. All correlations are highly significant and the 95 percent confidence limits for all comparisons except those involving the initial shrink were within approximately ± 3°C. The data obtained on different leathers and on raw hide substance suggest that readings taken after the specimens have shrunk a fraction of their length (five to ten percent) would have smaller confidence limits and a high degree of correlation to the absolute value for the thermal transition as measured by the peak temperature on DTA thermograms. Measurements on suspended specimens simulate more closely test conditions as conducted in tannery practice than measurements under tension and eliminate the need for cumbersome equipment and tedious arrangement of specimens.

ACKNOWLEDGMENT

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